J005

GENETIC AND NON-GENETIC FORMS OF ANEURYSMS OF THE HUMAN ASCENDING AORTA SHARE ACTIVATION AND OVEREXPRESSION OF SMAD2: PUTATIVE IMPLICATION OF EPIGENETIC MECHANISMS

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Ascending aortic aneurysm (AscAA) development proceeds by multifactorial and chronic processes affecting both vascular extracellular matrix structure and integrity and smooth muscle cell (SMC) survival. These features are associated with all types of AscAA: i) genetic forms associated with mutations in FBN1, TGFBR1 or TGFBR2 (Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS)), ii) aneurysms associated with bicuspid aortic valve (BAV) or iii) degenerative forms. However, the relation between the genotypic variability and the unique aortic phenotype remains unexplained. The common matrix perturbations suggest similar cell dysfunction in both genetic and non-genetic AscAA. In this context, it has been recently proposed that TGF-β1 plays a predominant role in AscAA. Here we investigate TGF-β1 and its intracellular mediator Smad signaling pathway using tissue extracts and cultured SMCs from the media of genetic and non-genetic forms of AscAA. We show an increased activation of Smad2 (phosphorylated-Smad2) and an increased amount of TGF-β1 in AscAA. However, biochemical and histological studies demonstrated an enhancement of TGF-β1 retention within the extracellular matrix but not in its expression and activation, and thus highlight independent dysregulation of TGF-β1 retention and Smad2 signaling in genetic and non-genetic aneurysms. The constitutive Smad2 activation is independent of the extracellular TGF-β1 as well as of TGF-β receptor functionality. Aneurysms bearing TGFBR2 mutations, which induce loss of function, present constitutive Smad2 activation. Moreover, increased Smad2 expression is observed in tissue extracts but also in cultured SMC extracts, where the overexpression is surprisingly maintained during several passages, in AscAA. The study of adventitial fibroblasts shows that Smad2 perturbations are specific to SMCs from the media of aneurysmal aortic wall. A putative regulation of Smad2 expression by epigenetic mechanisms (histone acetylation and/or DNA methylation) is tested. Preliminary results show decreased Smad2 expression induced by deacetylase and methylase inhibitors in aneurysmal SMCs. In contrast, Smad2 expression, in control SMCs, is not affected by these treatments. The constitutive and tissue-specific activation of Smad2 and its maintained expression suggest an implication of epigenetic mechanisms in the development of genetic and non-genetic AscAA.

J006

BETA-ADRENERGIC STIMULATION ACTIVATES PROTEIN KINASE C-EPSILON THROUGH EPAC IN CARDIOMYOCYTES

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Protein kinase C (PKC) activation is classically considered as independent of the β-adrenergic pathway. However, the cAMP-activated exchange factor Epac was recently shown to activate phospholipase C. β-A stimulation is thus likely to stimulate PKC. We evaluated in cardiomyocytes whether βA stimulation could activate PKCε. Rat neonatal cardiomyocytes were subjected to isoproterenol stimulation (ISO). Inositol trisphosphate production was increased by 50% by 1 μm ISO ($p<0.05$) and PKCε was translocated to particulate fractions (western blot) in the perinuclear area (confocal microscopy) in a PKA-independent manner since it was not inhibited by an infection with an adenovirus encoding a protein kinase A (PKA) inhibitor. Instead, PKCε activation was Epac dependent since 8-CPT, an Epac activator, induced the same PKCε translocation as ISO and siRNAs of Epac completely inhibited PKCε activation. The same translocation of PKCε in PF induced by βA stimulation was found in adult isolated rat hearts perfused by ISO with a sarcolemmmal membrane localization. This was associated with a phosphorylation of connexin-43 on ser368 that was blocked by the PKC inhibitor BIM. In conclusion, these data demonstrate a new interconnection between β-adrenergic and PKC pathways via Epac in cardiac cells with a potential role in cell-to-cell communications.

J007

CARDIAC REMODELING FOLLOWING CHRONIC ACTIVATION OF THE NOTCH SIGNALING PATHWAY

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The Notch signaling pathway is a communication system between adjacent cells, mediated by transmembrane receptors (Notch1-4) and ligands (Jagged1 and 2, Delta-like1, 3, and 4). Notch is essential for the development and homeostasis of several self-renewing organ systems. Notch is also essential in the developing heart, and mutations in Notch genes cause cardiac malformations and congenital heart disease. Recently, using loss-of-function studies, we showed that the Notch1 receptor controlled the response to injury in the adult heart by limiting myocyte hypertrophy, enhancing myocyte survival, promoting precursor proliferation, controlling cardiogenic differentiation, and reducing interstitial fibrosis. In addition, our data suggested that upregulation of Jagged1 expression constituted a primary response in the stressed myocardium, suggesting that this ligand mediated Notch signaling in the postnatal heart. Therefore, to analyze the effects of a chronic Jagged1-induced Notch activation on the cardiac response to stress, we generated transgenic mice overexpressing Jagged1 in cardiomyocytes using the α-myosin heavy chain gene promoter (TGMHCJ1 mice). These mice expressed 100-500 fold Jagged1 mRNA relative to wild-type (WT) controls. The overexpression of Jagged1 on the surface of cardiomyocytes was confirmed by Western blotting and immunofluorescence staining. Consequently, the percentage of cardiac cells with an activated Notch pathway was higher in TGMHCJ1 mice than in WT animals. The expression of the Notch target genes Hes1 and Hey2 was also activated. Echocardiographic analysis under basal conditions revealed an enlargement of the right ventricle (RV) with a diminished left ventricle (LV) mass and chamber size in adults. TGMHCJ1 mice displayed increased juvenile mortality. The severity of the phenotype was dependent on the level of Jagged1 expression. Immunofluorescence analysis revealed myocyte hypertrophy and disarray in the RV of TGMHCJ1 mice whereas, myocytes in the LV were